WEININGER WORKS™ ANALYTICS TESTS

Double Blind Tests

The analytic capablities of Susan Weininger remain unsurpassed.
Testing of analytics is accomplished by performing double-blind studies.

In carefully supervised double-blind tests Susan Weininger:

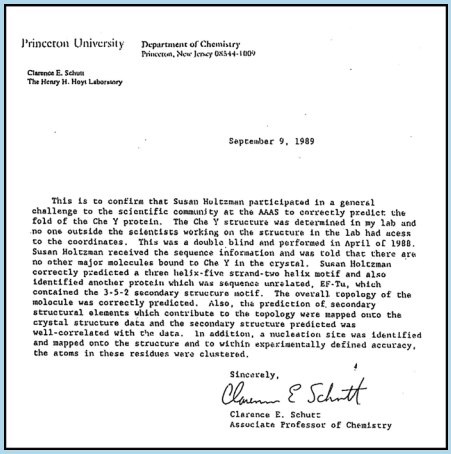
- has folded proteins to within experimentally defined accuracy from non-homologous sequence only; and
- has been able to determine how crystal structures differ from solution structures (i.e. crystallization condition artifacts).

At the time of the cheY and diphtheria toxin structure tests by Susan Weininger/Holtzman/Kilkowski, the cheY and diphtheria toxin sequences had no known homology to any other protein whose structure was solved but not released or known, respectively. A theoretical understanding of the intermolecular forces that determine the folding of a stable folded structure is necessary to evaluate (among other things):

- whether a crystal structure feature is present due to crystallization conditions (i.e. present or not present in solution);
- the stability of protein substructures and the protein itself;
- the effect of mutations; and
- the stabilizing or destabilizing effect of grafting or ligating functionality.

We believe that AI cannot independently get to a theoretical understanding of protein folding at this time or in the near future. The examples provided herein of our work are not comprehensive and are provided simply to provide an idea of the depth and breadth of our work. We have not included information related to ongoing discoveries, analyses, unpatented inventions, and any historical commercially-directed work.

Double Blind Tests



Princeton University Department of Chemistry
Princeton, New Jersey 08544-1009

Clarence E. Schutt
The Henry H. Hoyt Laboratory

September 9, 1989

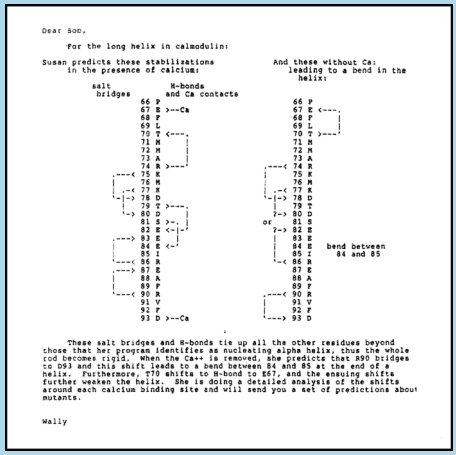
This is to confirm that Susan Holtzman participated in a general challenge to the scientific community at the AAAS to correctly predict the fold of the Che Y protein. The Che Y structure was determined in my lab and no one outside the scientists working on the structure in the lab had access to the coordinates. This was a double blind and performed in April of 1988. Susan Holtzman received the sequence information and was told that there are no other major molecules bound to Che Y in the crystal. Susan Holtzman correctly predicted a three helix-five strand-two helix motif and also identified another protein which was sequence unrelated, EF-Tu, which contained the 3-5-2 secondary structure motif. The overall topology of the molecule was correctly predicted. Also, the prediction of secondary structural elements which contribute to the topology were mapped onto the crystal structure data and the secondary structure predicted was well-correlated with the data. In addition, a nucleation site was identified and mapped onto the structure and to within experimentally defined accuracy, the atoms in these residues were clustered.

Sincerely,

Clarence E. Schutt

Clarence E. Schutt
Associate Professor of Chemistry

CheY documents (PDF)



Dear Sam,

For the long helix in calmodulin:

Susan predicts these stabilizations in the presence of calcium:

salt bridges	H-bonds and Ca contacts
	66 P
	67 E >--Ca
	68 F
	69 L
	70 T <---.
	71 M |
	72 M |
	73 A |
	74 R >---'
.----<	75 K |
|	76 M |
| .-<	77 K |
'-|->	78 D |
|	79 T >---.
'->	80 D |
	81 S >-. |
	82 E <-|-'
.--->	93 E |
|	84 E <-'
|	85 I
'----<	86 R
.--->	87 E
|	88 A
|	89 F
'----<	90 R
	91 V
	92 F
	93 D >--Ca

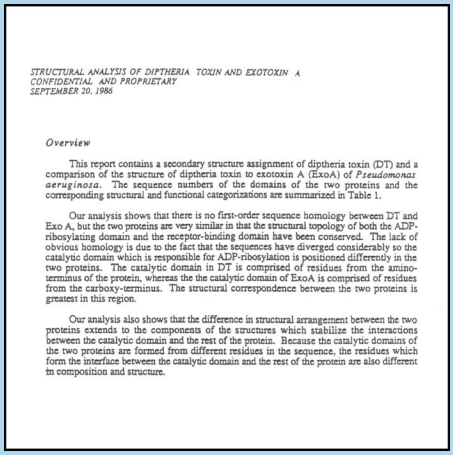
And these without Ca: leading to a bend in the helix:

66 P	
67 E <---.	
68 F |	
69 L |	
70 T >---'	
71 M	
72 M	
73 A	
.----< 74 R	
| 75 K	
| 76 M	
| .-< 77 K	
'-|-> 78 D	
| 79 T	
?-> 80 D	
or 81 S	
?-> 82 E	
83 E	
84 E	bend between
85 I	84 and 85
'-< 86 R	
87 E	
88 A	
89 F	
.----< 90 R	
91 V	
92 F	
'----> 93 D	

These salt bridges and H-bonds tie up all the other residues beyond those that her program identifies as nucleating alpha helix, thus the whole rod becomes rigid. When the Ca++ is removed, she predicts that 890 bridges to D93 and this shift leads to a bend between 84 and 85 at the end of a helix. Furthermore, 870 shifts to H-bond to E67, and the ensuing shifts further weaken the helix. She is doing a detailed analysis of the shifts around each calcium binding site and will send you a set of predictions about mutants.

Wally

Calmodulin documents (PDF)



STRUCTURAL ANALYSIS OF DIPTHERIA TOXIN AND EXOTOXIN A
CONFIDENTIAL AND PROPRIETARY
SEPTEMBER 20, 1986

Overview

This report contains a secondary structure assignment of diptheria toxin (DT) and a comparison of the structure of diptheria toxin to exotoxin A (ExoA) of *Pseudomonas aeruginosa*. The sequence numbers of the domains of the two proteins and the corresponding structural and functional categorizations are summarized in Table 1.

Our analysis shows that there is no first-order sequence homology between DT and Exo A, but the two proteins are very similar in that the structural topology of both the ADP-ribosylating domain and the receptor-binding domain have been conserved. The lack of obvious homology is due to the fact that the sequences have diverged considerably so the catalytic domain which is responsible for ADP-ribosylation is positioned differently in the two proteins. The catalytic domain in DT is comprised of residues from the amino-terminus of the protein, whereas the the catalytic domain of ExoA is comprised of residues from the carboxy-terminus. The structural correspondence between the two proteins is greatest in this region.

Our analysis also shows that the difference in structural arrangement between the two proteins extends to the components of the structures which stabilize the interactions between the catalytic domain and the rest of the protein. Because the catalytic domains of the two proteins are formed from different residues in the sequence, the residues which form the interface between the catalytic domain and the rest of the protein are also different in composition and structure.

DT documents (PDF)

CheY structure known but not released

In April 1988, Susan Weininger† successfully competed in a double blind AAAS protein structure general challenge. Susan Weininger predicted the structure of CheY to experimentally defined accuracy from sequence only. CheY had no sequence homology to any proteins with known structures.[1]

Calmodulin structure with artifacts related to solution

In February 1987, Susan Weininger† critiqued an x-ray structure of calmodulin for Walter Gilbert (Harvard University). Susan Weininger predicted the differences between the crystal structure and the solution structure of calmodulin. Susan Weininger predicted that the central helix in the crystallographic calmodulin structure (the “dumbell” structure) was not structured in solution. Not matching crystallographic positions, the central helix was not graded as “correct” until later solution studies[2, 3] confirmed Susan Weininger's analysis.

DT toxin with no known structure

In September 1986, Susan Weininger† correctly determined the structural correspondence between the ADP-ribosylating enzymes Exotoxin A and diphtheria toxin. There was no sequence homology between the proteins. Only the structure of Exotoxin A[4] was known. Susan Weininger correctly identified the glutamic acid in the diphtheria toxin active site. This was later confirmed by photoaffinity labeling. The structure of diphtheria toxin[5] would be published nearly six years later confirming the structural correspondences between Exotoxin A and diphtheria toxin.

1 Stock AM, Mottonen JM, Stock JB, Schutt CE
Three-dimensional structure of CheY, the response regulator of bacterial chemotaxis.
Nature 1989 Feb 23; (6209):745-749.
DOI: 10.1038/337745a0
PMID: 2645526

2 Persechini A, Kretsinger RH The central helix of calmodulin functions as a flexible tether.
J Biol Chem 1988 Sep 5; 263(25):12175-12178.
DOI: 10.1016/S0021-9258(18)37733-0
PMID: 3137220

3 O'Neil KT, Erickson-Viitanen S DeGrado WF
Photolabeling of calmodulin with basic, amphiphilic alpha-helical peptides containing p-benzoylphenylalanine.
J Biol Chem1989 Aug 25; 264(24):14571-14578
DOI: jbc.org/article/S0021-9258(18)67441-1/pdf
PMID: 2760074

4 Allured VS, Collier RJ, Carroll SF, McKay DB
Structure of exotoxin A of Pseudomonas aeruginosa at 3.0-Angstrom resolution.
Proc Natl Acad Sci U S A. 1986 Mar;83(5): 1320-1324. DOI: 10.1073/pnas.83.5.1320
PMID: 3006045

5 Choe S, Bennett MJ, Fujii G, Curmi PM, Kantardjieff KA, Collier RJ, Eisenberg D
The crystal structure of diphtheria toxin.
Nature 357 (6375), 216-222 (1992)
DOI: 10.1038/357216a0
PMID: 1589020

†Susan Kilkowski (prior to June 1985) = Susan Holtzman (June 1985 – July 1993) = Susan Weininger (July 1993 to present)